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TITLE: Pathogenesis and Prediction of Future Rheumatoid Arthritis

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CONTRACTING ORGANIZATION: University of Colorado Denver, Aurora, CO 80045

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14. ABSTRACT It is now well established that there is a preclinical period of rheumatoid arthritis (RA) development that is characterized by abnormalities of the immune system prior to the onset of the clinically apparent inflammatory joint disease that currently defines RA. The primary goal of this project is to investigate this preclinical period in order to understand two major factors: 1) how biomarker changes in preclinical RA can be used to accurately predict the future development of RA in currently asymptomatic individuals, and 2) to identify factors related to the pathogenesis of RA that can ultimately be targeted to prevent RA. This project has proposed to use a unique set of serum samples and clinical data available through the Department of Defense Serum Repository (DoDSR) to investigate the preclinical period of RA. During the first year of this project (30 Sep 2013-29 Sep 2014) we have acquired the serums samples and data from the DoDSR, and performed initial biomarker testing.					
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PI Deane, Kevin D.

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INTRODUCTION:

This is the first Annual Report for the project entitled “Pathogenesis and Prediction of Rheumatoid Arthritis”, PI Kevin D. Deane, period 30 Sep 2013 – 29 Sep 2014. The date that this report is submitted is 29-Oct-2014.

It is now well established that there is a preclinical period of rheumatoid arthritis (RA) development that is characterized by abnormalities of the immune system prior to the onset of the clinically apparent inflammatory joint disease that currently defines RA. The primary goal of this project is to investigate this preclinical period in order to understand two major factors: 1) how biomarker changes in preclinical RA can be used to accurately predict the future development of RA in currently asymptomatic individuals, and 2) to identify factors related to the pathogenesis of RA that can ultimately be targeted to prevent RA.

This project has proposed to use a unique set of serum samples and clinical data available through the Department of Defense Serum Repository (DoDSR) to investigate the preclinical period of RA.

As described below in more detail, during the first year of this project, we have acquired the serums samples and data from the DoDSR, and performed initial biomarker testing.

KEYWORDS:

Pathogenesis of rheumatoid arthritis
Prediction of rheumatoid arthritis

ACCOMPLISHMENTS:

What were the major goals of the project?

For this period of the project (30 Sep 2013 – 29 Sep 2014), as stated in the Statement of Work (SOW) the major goals were as follows:

- 1) Clinical data and sample procurement from the DoDSR. (SOW Task 1)
- 2) Obtain regulatory IRB/HRPO approval. (SOW Task 2)
- 3) Research assistant hiring and training. (SOW Task 3)
- 4) RA-related autoantibody testing in 1600 serum samples from 200 RA cases and 200 healthy controls. The specific autoantibodies include testing for anti-cyclic citrullined peptide (anti-CCP)-2, anti-CCP3.1, rheumatoid factor isotypes, and an array for antibodies to citrullinated protein antigens (ACPAs). (SOW Task 4)
- 5) Testing antibodies to oral pathogens in 1600 serum samples from 200 RA cases and 200 healthy controls. (SOW Task 6).

What was accomplished under these goals?

Goal 1. We obtained clinical data and serum samples from the DoDSR. These samples are now housed at the University of Colorado Denver in Dr. Deane’s lab and the serum samples have used to perform the tests as listed below. Clinical data was obtained per

medical chart review and includes the items listed in the original SOW as follows: subject age, gender, race, region of enlistment and military specialty, time of onset of RA and symptoms, classification criteria met for RA, medication use pre and post-RA diagnosis. In addition, other medical illnesses and other environmental exposures such as smoking, periodontal disease were ascertained.

Goal 2. Local and governmental IRB approvals, and HRPO approval, were obtained for this project.

Goal 3. Research assistants were hired and trained for this project. Please see the 'Participation' section below for details of these individuals.

Goal 4. We performed serum sample testing for each of the following:

- Anti-CCP2 using ELISA kits (Axis-Shield) and established methodologies.
- Anti-CCP3.1 using ELISA kits (INOVA) and established methodologies.
- Rheumatoid factor isotypes (A, G and M) using ELISA kits (INOVA) and established methodologies.
- Samples for testing of ACPA arrays using bead-based array and established methodologies have been shipped to lab of co-investigators Drs William Robinson and Jeremy Sokolove. Completion of this testing was delayed due to delays in obtaining reagents; however, materials are obtained and testing will be complete by 30 Nov 2014.

To date to preserve the scientific integrity of the project, we are remaining blinded to subject status (e.g. RA case v. control), and full un-blinded analyses of these results are planned for years 2 and 3 of this project. However, in preliminary unblinded analyses we have found that a substantial proportion of samples were positive for autoantibodies (see the Table immediately below). Overall, these results demonstrate that the sample set obtained from the DoDSR will have sufficient autoantibody positivity and are adequate to accomplish the overall goal of the project which is to use biomarkers and other factors to develop a model to predict the likelihood and timing of future RA.

Preliminary Results of Autoantibody Testing of 1600 Samples and Blinded to RA Case v. Control Status	
Autoantibody Test	% positive (of 1600 samples)
CCP3.1	497 (31%)
IgA-RF	235 (15%)
IgM-RF	487 (30%)
IgG-RF	448 (28%)
Any Ab	608 (38%)

Goal 5. We have completed testing for oral pathogens *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusibacterium nucleatum*.

This was performed in the lab of Drs Ted Mikuls and Geoffrey Thiele using established protocols. Full analyses of these results are planned for years 2 and 3 of this project.

What opportunities for training and professional development has the project provided? In this initial phase of the project, activities did not include training and professional development. However, in years 2 and 3 of the project we expect that this project will allow for early career rheumatologists including fellows and junior faculty to participate in scientific research. During the next two years of the project, these activities will expand through more detailed analyses and publications, and additional personnel will be posted when they become known.

How were the results disseminated to communities of interest? Nothing to report because during this first year of the three-year project, our goals were to perform data and sample acquisition, and initial sample testing.

What do you plan to do during the next reporting period to accomplish the goals? During the next two years of the project, we will complete sample testing, and then perform analyses as described in our Statement of Work.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project? Nothing to report at this phase of the project.

What was the impact on other disciplines? Nothing to report at this phase of the project.

What was the impact on technology transfer? Nothing to report at this phase of the project.

What was the impact on society beyond science and technology? Nothing to report at this phase of the project.

CHANGES/PROBLEMS:

Changes in approach and reasons for change There have been no substantive changes in the overall approach. However, we had initially allocated a certain amount of funds to obtain the serum samples from the DoDSR; however, we needed fewer funds than anticipated. As of October 2014 we have therefore petitioned the DoD to use these remaining funds for additional testing of the serum samples; specifically to test for isotypes immunoglobulin (Ig) A, IgG and IgM to citrullinated proteins. We are currently awaiting a final decision from the DoD whether those funds will be available. We will update the SOW and other materials once this additional testing has been approved.

Actual or anticipated problems or delays and actions or plans to resolve them Due to the government shut-down and then funding cut-backs at the DoD level, the process of extracting data and serum samples from the DoDSR was delayed; however, we have obtained the samples and are on track to complete the proposed testing as outlined in the Statement of Work. Also, as mentioned above, there was a delay in obtaining reagents for

ACPA array testing; these reagents have now been obtained and this testing will be complete by 30 Nov 2014.

Changes that had a significant impact on expenditures Please see 'Changes in Approach' above.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents Nothing to report.

Significant changes in use or care of human subjects Nothing to report.

Significant changes in use or care of vertebrate animals Nothing to report.

Significant changes in use of biohazards and/or select agents Nothing to report.

PRODUCTS:

Publications, conference papers, and presentations All analyses and resultant publications are planned for years 2 and 3 of this project; as such at this time, there is nothing to report.

Journal publications Nothing to report.

Books or other non-periodical, one-time publications Nothing to report.

Other publications, conference papers, and presentations Nothing to report.

Website(s) or other Internet site(s) Nothing to report.

Technologies or techniques Nothing to report.

Inventions, patent applications, and/or licenses Nothing to report.

Other Products Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project? There are no changes related to the original investigators on the project as reported in the original proposal with the exception that research assistants have been named for the project. The investigators and research assistants are as follows (and listed in alphabetical order after the PI).

Name	Kevin D. Deane, MD/PhD
Project Role	PI
Nearest Person Month Worked	2
Contribution	Oversee the entire project
Funding Support	This project

Name	Jess Edison, MD
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Data extraction; sample management at DoD
Funding Support	Dr. Edison is paid through his position as active duty military and receives no funds from this project.

Name	V. Michael Holers, MD
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Oversee biomarker testing
Funding Support	This project

Name	Ted R. Mikuls, MD/MSPH
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Testing for antibodies to oral pathogens
Funding Support	This project

Name	William Robinson, MD/PhD
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Testing ACPA array
Funding Support	This project

Name	Jeremy Sokolove, MD
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Testing ACPA array
Funding Support	This project

Name	Geoff Thiele, PhD
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Testing for antibodies to oral pathogens
Funding Support	This project

Name	Gary O. Zerbe, PhD
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Statistical analyses; study design and power
Funding Support	This project

Name	Marie Feser, MSPH
Project Role	Study Coordinator
Nearest Person Month Worked	12
Contribution	Oversee the entire project
Funding Support	This project

Name	Mark Parish, BA
Project Role	Research assistant/laboratory technician
Nearest Person Month Worked	12
Contribution	Laboratory testing/sample management
Funding Support	This project

Name	Emily Stein, PhD
Project Role	Research assistant
Nearest Person Month Worked	3
Contribution	ACPA testing
Funding Support	This project

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Please see the following table.

Changes in Active Other Support for PI/Key Personnel During the Reporting Period 30 Sept 2014 – 29 Sept 2015	
Kevin Deane, MD/PhD	Dr. Deane has received an appointment at the Denver Veterans Affairs Hospital and received new NIH grant funding; these changes do not affect his effort on this project.
Jess Edison, MD	No changes.
V. Michael Holers, MD	Dr. Holers has received new NIH grant funding and had one grant expire; these changes do not affect his effort on this project.
Ted Mikuls, MD/MSPH	No changes.
William Robinson, MD/PhD	Dr. Robinson has received new NIH funding; these changes do not affect his effort on this project.
Jeremy Sokolove, MD	Dr. Sokolove has received new NIH funding; these changes do not affect his effort on this project.
Geoffrey Thiele, PhD	No changes.
Gary O. Zerbe, PhD	No changes.

What other organizations were involved as partners? There have been no changes from the original proposal in the organizations involved in this project. The organizations that have participated in this project are as follows:

University of Colorado Denver

1775 Aurora Court

Aurora, Colorado USA

The PI Dr. Deane and co-investigator Dr. Holers are based at this institution; all data and samples are housed at this institution.

University of Nebraska Medical Center

986270 Nebraska Medical Center

Omaha, NE 68198 USA

Co-investigators Drs Mikuls and Thiele are based at the University of Nebraska and are performing the testing for antibodies to oral pathogens as well as contributing to the overall design and implementation of the project.

Veterans Affairs Palo Alto Health Care System

3801 Miranda Avenue

Palo Alto, CA 94304

Co-investigators Drs Robinson and Sokolove are based at the Palo Alto VA and are performing testing for the ACPA array and related analyses.

Walter Reed National Military Medical Center

8901 Wisconsin Avenue

Bethesda, MD 20889

Co-investigator Dr. Edison is based at Walter Reed and is obtaining the clinical data and military IRB approvals related to this project.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not applicable.

QUAD CHARTS:

Not applicable.

APPENDICES

None.